ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 67 ART. 7 PAGES 185-206

Editor in Chief
OTTO V. ST. WHITELOCK

Managing Editor
FRANKLIN N. FURNESS

Associate Editor
MARGARET P. CAMERON

THE USE OF CERTAIN SULFONAMIDES IN THE TREATMENT OF EXPERIMENTAL DIABETES MELLITUS

By

AUGUSTE LOUBATIÈRES

NEW YORK
PUBLISHED BY THE ACADEMY
December 28, 1956

Copyright, 1956, by The New York Academy of Sciences

THE USE OF CERTAIN SULFONAMIDES IN THE TREATMENT OF EXPERIMENTAL DIABETES MELLITUS

By

Auguste Loubatières

Laboratory of Applied Physiology, Faculty of Medicine,
Institute of Biology, Montpellier, France

Early Clinical Observations

The first clinical and experimental observations that form the basis for the use of certain sulfonamides in the treatment of diabetes mellitus were made early in 1942. At that time, Janbon and his co-workers noticed fortuitously during therapeutic experiments that a sulfonamide, *p*-aminobenzenesulfonamidoisopropylthiodiazole (VK 57, or 2254 RP, or IPTD), as illustrated in FIGURE 1, produced disorders very similar to hypoglycemia. In the most serious cases these were followed by irreversible or fatal nervous symptoms, particularly in patients suffering from typhoid fever, who were underfed and consequently predisposed to hypoglycemia.

Because of the nature of these symptoms, Janbon consulted me, and since that time, both at the Clinique des Maladies Contagieuses and at the Physiology Laboratories of the Faculté de Médecine de Montpellier, we have been engaged in determining the nature of these symptoms and analyzing the mechanism by which they are produced.

Mechanism of Action

The symptoms were similar to those occurring during acute insulin hypoglycemia. Although the symptoms of acute insulin hypoglycemia had been well-known for 20 years, we had only recently obtained experimental evidence of the consequences of prolonged hypoglycemia produced by excessive doses of insulin. In 1939, in collaboration with L. Hédon and C. Heymann (Hédon, Loubatières, and Heymann, 1939), I had described the irreversible nervous disorders observed in the pancreatectomized dog treated with protamine zinc insulin. Although glucose injections were able to reverse acute or recent hypoglycemia, they were ineffective if hypoglycemia had been present for 12 hours or more. Although the hypoglycemia itself disappeared, the convulsive fits continued and were sometimes even aggravated by the administration of glucose. The lesions of the nerve centers were irreversible except in a few cases where high doses of vitamin B_1 could cause them to regress if an adequate glucose-vitamin treatment were started in time.

Janbon had observed that the administration of 2254 RP in man produced hypoglycemic symptoms that were reversible if glucose injections

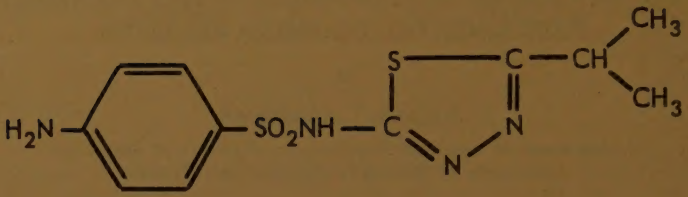

FIGURE 1. *p*-Aminobenzenesulfonamidoisopropylthiodiazole (2254 RP).

were given in time. If this treatment was carried out too late, however, permanent or fatal nervous disorders occurred.

By carrying out animal experiments we attempted to establish the parallel that seemed to exist between the actions of 2254 RP and insulin. In June 1942, we were able to assure Janbon that in acute experiments the oral administration of 2254 RP to a fasting and conscious dog caused a progressive and lasting hypoglycemia, and that a dose of 0.25 gm./kg. *per os* was sufficient to bring the blood-glucose level down to 0.50 gm./liter. Furthermore, the degree of hypoglycemia appeared to be a function of the blood sulfonamide level: the maximum hypoglycemia and the maximum blood sulfonamide level occurred simultaneously and, the day after the experiment, the blood-glucose level was always lower than that of the previous day at the same time. I repeated these experiments and obtained the same results. In chronic experiments, also carried out in June 1942, the oral administration of 2254 RP for several days produced a hypoglycemia that was most evident when the blood sulfonamide level was highest.

At the same time, and on numerous later occasions, I showed that in totally depancreatized dogs oral administration of 2254 RP had no appreciable effect on the glucose level. The drug was also ineffective when administered intravenously or intramuscularly in sodium hydroxide solution. I therefore put forward the hypothesis that 2254 RP acted on the islets of Langerhans, stimulating insulin secretion or increasing the effects of insulin itself.

Janbon and his colleagues submitted a summary of their clinical observations to the Société des Sciences Médicales et Biologiques de Montpellier (Janbon *et al.*, 1942; Janbon, Lazerges, and Metropolitanski, 1942). My experiments are mentioned on page 443 of the report. The full text appeared very much later in a collection of papers from the Clinique des Maladies Contagieuses, of which Janbon is director. Reference to my experiments may be found on page 220 of the latter publication.

In July 1942, by oral and parenteral administration of large doses of 2254 RP to dogs, we were able to produce nervous symptoms that could not be reversed by glucose. In October 1942, we (Loubatières *et al.*, 1942) reported these findings to the 43rd Congress of French Psychiatrists and Neurologists. At the same meeting, Janbon and his colleagues (Janbon, Chaptal, and Vedel, 1942) described the irreversible nervous disorders produced in man by 2254 RP. They adopted the pathogenic interpretation resulting from our experiments.

In May 1943, we showed that the hypoglycemic action of 2254 RP occurred in a dog whose pancreas had been reduced to one sixth of its initial mass, when the remaining glandular vestige was in the region where the principal pancreatic canal opens into the duodenum. The values for blood-glucose and blood-sulfonamide concentrations varied inversely. In one experiment, where a 0.3-gm. fragment of pancreas was accidentally left about the main pancreatic canal, we observed that, whereas the animal was obviously hyperglycemic when fasting, the oral administration of 2254 RP brought down the sugar level. This compound also increased the respiratory quotient of the same animal if glucose was administered simultaneously by mouth. In contrast, in a totally depancreatized dog, which does not normally react to glucose administration by increasing its respiratory quotient, 2254 RP did not increase the respiratory quotient, whether administered alone or with glucose. These and subsequent experiments showed, therefore, that the hypoglycemic effect of 2254 RP could occur only when pancreatic tissue was present in the body.

My hypothesis that 2254 RP stimulates the secretion of insulin seemed to be confirmed, but the mechanism remained to be proved. To test the possibility that the stimulus was transmitted to the pancreas by the pneumogastric nerves, I administered 2254 RP to a dog sufficiently atropinized to stop the effects of acetylcholine injection, and found that the hypoglycemic action of 2254 RP was unaffected. It also occurred with the same intensity after section of the 2 pneumogastric nerves (April 1946). It seemed, therefore, that 2254 RP acted humorally.

To prove this hypothesis of humoral action, I have shown, on the one hand, that the slow injection of a small dose of 2254 RP (0.04 gm./kg.) into the pancreatic artery draining the uncinate process of the dog brought about a fall in the blood-sugar level; on the other hand, the injection of a sodium hydroxide solution of 2254 RP (0.04 gm./kg.) in the glandular end of the main pancreatic canal after ligature of its duodenal end, produced, after a short hyperglycemic phase, a pronounced hypoglycemia in which the blood-glucose level could drop as low as 0.30 gm./liter. I had tentatively attributed this initial hyperglycemia to excitement of the splanchnic nerves when preparing the pancreatic canal, but considered the possibility that the release of insulin by the islets of Langerhans occurred only after a phase of inhibition. I also emphasized that intravenous injection of alloxan in diabetogenic doses produces, in both dogs and in rabbits, comparable glycemic modifications and variations (Loubatières, 1946). Only in the last year have we (Loubatières, Bouyard, and Fruteau de Laclos, 1954, 1955a and b) established the true explanation, that any liquid injection directed toward the glandular tissue via the pancreatic excretor canals can immediately stimulate the islets of Langerhans.

In 1946, we were able to prove (Loubatières, 1946c) that 2254 RP caused the release of insulin. We did this by carrying out a pancreatic-jugular anastomosis (Zunz and La Barre method) between the duodeno-pancreatic vein of a donor dog and the jugular vein of a transfused dog previously rendered diabetic by alloxan.

It was to be expected that the administration of 2254 RP to a normal dog would produce metabolic responses comparable to those induced by insulin. In fact, we were able to demonstrate that this compound promoted the accumulation of large quantities (up to 10 times the initial amount) of glycogen in the liver of a normal dog that had not been fed for 4 or 5 days (Loubatières, 1944a and b; 1946a and c). I interpreted the glycogenopexic action of 2254 RP as a secondary effect, due to the stimulation of insulin secretion. Our experiments also indicated that 2254 RP, unlike synthalin (decamethylenediguanidine) has no toxic effects on the hepatic cells, even in relatively large doses, since it is unlikely that they would be capable of storing glycogen if they had been injured (Loubatières, 1946a).

I speculated as to whether the hypoglycemic effect of 2254 RP might be explained by an inhibitory action on the specific effects of the "diabetogenic hormone" (somatotropic hormone) of the anterior lobe of the pituitary gland. In December 1944, however, I was able to show that 2254 RP did not affect the hyperglycemic action of a "diabetogenic" extract of bovine anterior hypophysis. On the contrary, during the diabetes produced by the injection of anterior pituitary extract in a normal dog the hypoglycemic effects of 2254 RP were reduced. This fact can be explained in the light of present knowledge.

I also wondered whether 2254 RP might be capable of activating the enzymatic processes occurring during glycolysis. I have shown *in vitro*, in heparinized dog blood, that at 37° C. and at a concentration of 1/5000, 2254 RP did not alter the normal glycolysis curve (Loubatières, 1946a and c). This concentration would correspond *in vivo* to a blood-glucose level of 0.5 to 0.6 gm./liter.

At this time I considered studying the effects of 2254 RP on alloxan diabetes, the characteristics of which I had recently described (Loubatières, 1946b). This condition is characterized by an almost selective lesion of the majority of the β cells of the islets of Langerhans. After a series of tests yielding variable results, I was able to carry out a precise and thorough experiment on 2 rabbits rendered diabetic by alloxan (Loubatières, 1946a). I reproduce the results here:

In January 1946 two rabbits of the same litter received the same treatment. One rabbit weighed 2.350 kg.; the other, 2.460 kg. These animals received intravenously 150 mg. alloxan per kilogram of body weight. They were then put on a diet consisting solely of oats. Diabetes was established and urinary glucose varied between 14 and 28 gm. per day (FIGURE 2). From the 7th day of the experiment the 2 rabbits were not fed and, on the 10th day, without anesthesia, a fragment of pancreas was obtained aseptically for histological examination. Feeding was restored and, until the 25th day, the diet consisted of 75 gm. oats per day; then the diet was changed to 400 gm. cabbage daily. From the 18th to the 37th day, that is, during 20 days, each rabbit received 1 gm. 2254 RP in powdered form, mixed with its food. After this treatment with 2254 RP, the cabbage diet was continued until the 43rd day, after which 75 gm. oats were given again as in the earlier part of the experiment. On the 54th day, a second biopsy was carried out.

FIGURE 2 shows that 2254 RP given orally seemed to reduce the glycosuria caused by a diet rich in carbohydrates (oats). After the cabbage diet, the urinary glucose was also very low. When the administration of

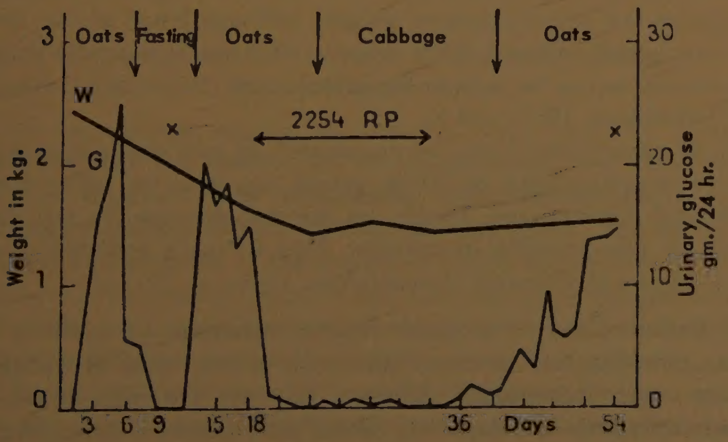

FIGURE 2. Rabbit with alloxan diabetes. Effect of oral administration of 2254 RP (1 gm./kg./day) on the degree of glycosuria G and on the weight *W*. The crosses indicate the time when the 2 biopsies were performed. Temporary cure of diabetes. (Loubatières, 1946*a*.)

2254 RP was discontinued, diabetes reappeared. Consequently it seemed that the *p*-aminobenzenesulfonamidoisopropylthiodiazole enabled these 2 animals to utilize the carbohydrates. This finding is consistent with the fact that the weight of both animals was stable henceforward. During the administration of 2254 RP the appetite of the animals was rather erratic – a factor that might contribute to the decrease in urinary glucose but was obviously not the reason for the weight stabilization. It is reasonable to suppose that 2254 RP increases the carbohydrate tolerance of the diabetic animal as long as a few active islets remain in the pancreas. In these 2 animals the presence of active islets was indicated by the fact that fasting stopped the glycosuria (9th to 12th day) and was proven by the aseptic biopsy performed at this time. When the sulfonamide treatment was discontinued the diabetes reappeared, progressed slowly, and attained its initial severity. Therefore, one should not attribute to 2254 RP the power of regenerating the islets of Langerhans. In fact, the second biopsy, carried out on the 54th day of the experiment, revealed the existence of occasional atrophied islets, a few of which were still degenerating. It is quite possible that the hyperglycemic state is opposed to the manifestation of any trophic action on the islets.

In 1946 I treated rabbits suffering from alloxan diabetes with 2254 RP and was surprised to find that some of them were completely cured of their diabetes (Loubatières, 1946b). I thought this must be a spontaneous late cure of diabetes after alloxan intoxication, and did not connect it with the action of 2254 RP. These results were very difficult to explain because the diabetes did not reappear on further administration of alloxan and because the animals gained weight, although I had shown that the islets were greatly altered. This question of a permanent cure continued to concern us, as can be seen in our publications (Hédon and Loubatières, 1949; Loubatières, 1952a and b).

These experiments could be considered as only preliminary to more extensive investigations on other animal species, on other forms of experimental diabetes and, finally, on the diabetic man. In any case, it was not too unreasonable to consider 2254 RP as a possible means of treating 2 forms of diabetes: (Loubatières, 1946a, p. 77)

(1) Diabetes due to sluggish insulin secretion. In addition to the diabetes resulting from anatomic alteration of the islets of Langerhans, there can occur "functional" diabetes, in which the cells of the islets of Langerhans seem histologically normal yet possess a raised excitation threshold and release less than the requisite amount of insulin.

(2) Diabetes complicated by infection. In this case the bacteriostatic action of the drug will make it especially valuable.

With regard to the toxicity of 2254 RP, acute and chronic tests on rabbits showed (Loubatières, 1946a) that the animals were able to tolerate relatively large doses of the compound without showing any symptoms of intoxication. This is further confirmed by the fact that 2254 RP promoted the storage of glycogen in the liver of the fasting dog, a proof that it did not injure the hepatic cells.

At the time this work was undertaken, knowledge of the part played by the α cells and by glucagon was lacking, and it was thought that simple hematein-eosin staining was sufficient to interpret the functional activity of the islets of Langerhans. Using this technique, Guibert and I made the following observations: (1) In alloxan-diabetic rabbits, a few active islets remained but, after treatment with 2254 RP, pancreatic biopsy showed the presence of rare atrophied islets, some of which had completely degenerated. (2) In normal rabbits, treated chronically (20 days) with 2254 RP (0.5 gm./kg./day orally), pancreatic biopsy showed hyperplastic islets of Langerhans: the cells had increased in volume and their nuclei were enlarged. The general condition of the animals was excellent.

Chen, Anderson, and Maze (1946) have studied a similar substance, 2-sulfanilamide-5-cyclopropyl-1,3,4-thiodiazole, which, given over a long period of time, produces a goiter. When administered to a normal rabbit, it produced severe hypoglycemia. In a rabbit with alloxan diabetes, however, it produced definite hyperglycemia. These authors quoted our work and suggested that the substance they used was also able to stimulate the secretion of insulin.

Pharmacodynamic Research: Relationship Between Structure and Activity

Bovet and Dubost carried out tests on dogs while we conducted tests on both dogs and rabbits. All the sulfonamides used were prepared for us by the Rhone-Poulenc laboratories. The chemical formulae, preparations, classification, effects on blood-glucose levels, and our personal conclusions can be found in the papers by Bovet and Dubost (1944) and Loubatières (1944a and b; 1946a, c, and d). Considering compounds structurally similar to *p*-aminobenzenesulfonamidoisopropylthiodiazole (2254 RP), we were able to demonstrate or confirm the following facts:

(1) Among the nonsulfonamide aminothiodiazoles tested (aminopropylthiodiazole, aminoisopropylthiodiazole, acetylaminoisopropylthiodiazole, benzoylaminoisopropylthiodiazole, and *p*-aminobenzoylaminoisopropylthiodiazole), none had a hypoglycemic action in dogs or rabbits. We were able, therefore, to draw the conclusion that the sulfonamide group is necessary to reduce the blood-sugar level (Bovet and Dubost, 1944; Loubatières, 1944b).

(2) Among the benzene sulfonamidothiodiazoles, the *p*-benzenesulfonamidoisopropylthiodiazole produced a moderate hypoglycemic effect in the dog (Bovet, personal communication).

(3) Among the *p*-aminobenzenesulfonamide thiazoles, *p*-acetylaminobenzenesulfonamidoundecylthiazole and *p*-aminobenzenesulfonamidoundecylthiazole were slightly hypoglycemic in the dog (Bovet and Dubost, 1944), but the latter compound had a *hyper*glycemic effect in the rabbit (Loubatières, 1944b).

I speculated whether it would be possible to affect the hypoglycemic potency of 2254 RP by varying the chemical structure of the side chain in position 5 of the thiodiazole nucleus. I studied 12 of these *p*-aminobenzenesulfonamide thiodiazoles in both dogs and rabbits (TABLE 1), and concluded that the tertiary butyl or isobutyl derivatives were the most active, followed, in order of decreasing activity, by the butyl, amyl, isoamyl and, finally, propyl and isopropyl derivatives. Methyl and ethyl derivatives were inactive, as were the hexyl, heptyl, and amino derivatives. Janbon *et al.* (1943), who investigated 5 of these products (isopropyl, methyl, ethyl, propyl, and isoamyl) in human beings, and Bovet and Dubost (1944), who tested 8 in the dog, reached almost the same conclusions. Among the *p*-aminobenzenesulfonamidoisopropylthiodiazole derivatives that we have systematically studied in rabbits, some were immediately hypoglycemic and others had a biphasic action, initially hyperglycemic and subsequently hypoglycemic. This biphasic action was not always clearly defined.

Even at this time, it was clear that both the portion of the molecule containing the thiodiazole nucleus and the nature of the hydrocarbon

TABLE 1

HYPOGLYCEMIC ACTIVITY OF *p*-AMINOBENZENESULFONAMIDOTHIODIAZOLES

Compounds tested *(0.25 gm./kg. subcutaneously, in the rabbit)*	*Percentage change relative to the initial level of blood glucose*
p-Aminobenzenesulfonamidomethylthiodiazole	+ 21
p-Aminobenzenesulfonamidoethylthiodiazole	− 14
p-Aminobenzenesulfonamidopropylthiodiazole	− 20
p-Aminobenzenesulfonamidobutylthiodiazole	− 30
p-Aminobenzenesulfonamidoisopropylthiodiazole	− 21
p-Aminobenzenesulfonamidoisobutylthiodiazole	− 38
p-Aminobenzenesulfonamido-*t*-butylthiodiazole	− 44
p-Aminobenzenesulfonamidoamylthiodiazole	− 30
p-Aminobenzenesulfonamidoisoamylthiodiazole	− 26
p-Aminobenzenesulfonamidohexylthiodiazole	− 5
p-Aminobenzenesulfonamidoheptylthiodiazole	− 14
p-Aminobenzenesulfonamidoaminothiodiazole	− 13

side chain in position 5 of this nucleus played an important part in the mechanism of the hypoglycemic action. Later work (Loubatières, 1946a, c, and d), confirmed by more recent research (Loubatières, Bouyard, and Fruteau de Laclos, 1955 unpublished data), showed that compounds having a structure similar to that of the thiodiazole side chain of 2254 RP had a depressant action on the sugar level in the blood. Thus, in the conscious dog, at a subcutaneous dose of 1 ml./kg., ethyl and methyl alcohols exerted no appreciable action on the blood glucose, whereas isopropyl, amyl, isoamyl, and butyl alcohols were hypoglycemic. The blood-sugar curves produced by these alcohols were similar to the curve from the corresponding sulfonamide compound. In 1946 (Loubatières, 1946a) I established a relationship among the blood-sugar curves obtained from the more hypoglycemic sulfonamide compounds, the corresponding alcohols, and a test dose of insulin. I have reported all these facts several times, and Houssay (1950) has reviewed this work.

In 1951, while studying the pathogenic role of "endogenous" alloxan, we (Loubatières and Bouyard, 1951; Loubatières, 1954) showed that 2254 RP *in vitro* prevented the formation of alloxanoid substances by the intestinal mucous cells, just as it prevents hyperalloxanemia induced in the fasting animal by the intestinal absorption of glucose or reduces the hyperalloxanemia observed in some forms of experimental diabetes.

Davis (1952) has shown that the subcutaneous injection of a solution of synthalin A (decamethylenediguanidine) sulfate or hydrochloride in the rabbit produces hydropic degeneracy of the α cells of the islets of Langerhans.

Von Holt and his colleagues conceived the idea of using *p*-aminobenzenesulfonamidoisopropylthiodiazole to determine whether this substance injured the α cells of the islets of Langerhans. They published a series of papers (von Holt *et al.*, 1954; von Holt *et al.*, 1955; von Holt and Ferner, 1955) without mentioning our previous work in their initial publication. These authors described the hypoglycemic action of *p*-aminobenzenesulfonamidoisopropylthiodiazole in rabbits and confirmed our finding that there was a sustained reduction of blood-sugar level. They also reported the beneficial action of this substance on alloxan diabetes in the rabbit, thus substantiating the effect we had described in 1946. They found (von Holt *et al.*, 1954) that *p*-aminobenzenesulfonamidoisopropylthiodiazole did not injure the hepatic cells, an observation that confirmed our early histological observations on the low toxicity of this substance.

By using the Gomori staining technique, von Holt and his co-workers observed important degenerative alterations of the α cells of the islets of Langerhans, accompanied by the disappearance of many cells. Evaluating the ratio of α to β cells, they found a marked decrease of α cells, whereas the β cells remained intact. On the other hand, in rabbits treated successively by alloxan and 2254 RP, von Holt and his colleagues noticed lesions of both the α and β cells that, in extreme cases, proceeded as far as the complete disappearance of the insular formation.

In 1955, in collaboration with my colleagues Bouyard and Fruteau de Laclos, I was able to reproduce and to confirm some of von Holt's histological findings. I was then in a position to explain the temporary cures of alloxan diabetes that we had observed in 1946. I had supposed at that time that 2254 RP acted only by stimulating insulin secretion. It was difficult to understand how this stimulation could occur since, after the administration of alloxan and 2254 RP, the islets were severely damaged. Yet there was an effective improvement in the general condition, as could be seen from the weight increase. We had also observed permanent cures of alloxan diabetes in rabbits after treatment with 2254 RP but, as I have mentioned earlier, we had postulated a spontaneous cure of the type that occasionally occurs in early alloxan diabetes or in pituitary diabetes.

Creutzfeldt and Tecklenborg (1955) have recently compared the effects of 2254 RP and synthalin on rabbits. They observed that the drug caused hypoglycemia in rabbits at a time when α-cell lesions were not apparent, but they suggested that this was probably due to the dose used. These investigators found the livers of the treated animals rich in glycogen. They showed that in a 2254 RP-treated rabbit injections of glucagon or adrenalin gave rise to hyperglycemia, whereas there was no reaction in the synthalin-treated rabbit (this glucagon-induced hyperglycemia was less, however, than in the normal control rabbit). Creutzfeldt and Tecklenborg believed that the α-cell lesions caused by large doses of 2254 RP were different from those due to synthalin.

It seemed likely that the hypoglycemia produced by the drug before the appearance of any α-cell lesion was due to a β-cell stimulation. This would agree with our first experiments and our interpretation of them. We thought then, on the basis of both our early and more recent experiments, that 2254 RP might act by an initial stimulation of the β cells (causing a release of insulin) and of the α cells of the islets of Langerhans. Using sufficiently large doses of 2254 RP, this temporary stimulation of the α cells would be followed by functional, then by structural, alterations of these cells, while the β cells remained unaffected. Either of two consequences might follow: an insulin release, with reduction of the blood sugar, or release of glucagon from the α cells. The effects of glucagon release might be evident under certain circumstances (as after intravenous injection or injection of 2254 RP into the main pancreatic canal) or might be obscured by the simultaneous release of insulin by the β cells.

When extensive and irreversible lesions of the α cells occurred, blood glucose would be mainly regulated by the β cells. There were then 2 possibilities: if the animal was normal before the administration of 2254 RP, hypoglycemia would occur and there would probably be relative sensitization to a determined amount of insulin; if the animal was diabetic through functional insufficiency or from β-cell lesions, the disorder caused by the functional prevalence of β cells might be counterbalanced (after 2254 RP) by functional alteration (temporary cure) or chronic or permanent lesion (lasting cure) of the α cells.

Since our previous work had convinced us that 2254 RP could injure the α cells severely and permanently, we undertook in January 1955 to show unequivocally that treatment with 2254 RP could effect a permanent cure of alloxan diabetes, not only in the rabbit but also in the dog (which is considerably more difficult). We were able to confirm and complete our previous experiments (Loubatières, Bouyard, and Fruteau de Laclos, 1955c, d, and e; Loubatières, 1955c, d, and e). A summary of these papers follows:

Hypoglycemic effect of 2254 RP on a normal animal. p-Aminobenzenesulfonamidoisopropylthiodiazole exhibited a hypoglycemic action in rats, rabbits, and dogs when it was administered orally, subcutaneously, intravenously, intramuscularly, or intraperitoneally. It had the same effect in the human being when given orally.

In the acute experiments, 0.4 gm. to 1 gm. 2254 RP per kg. was administered (orally, intravenously, or intramuscularly) to a normal dog 18 hours after the last meal. The blood-glucose level was reduced by from 30 to 60 per cent, reaching its minimum in 2 to 4 hours and remaining at a low value for 6 to 12 hours. The hypoglycemic manifestations could be cured by the administration of glucose. Twenty-four hours after the administration of 2254 RP the blood-glucose level was lower than at the same time on the previous day. The lowest blood-sugar level occurred when the sulfonamide level was highest (FIGURE 3).

In the chronic experiments, when dogs received 0.5 gm. of 2254 RP per kg. orally for several consecutive days, their blood-sugar levels were considerably lower than normal. This continued during the entire treatment. The sulfonamide level could be kept very high by the repeated administration of 2254 RP. After large doses of the drug, the animals sometimes showed nervous symptoms that were not reversed by the administration of glucose. These symptoms were comparable to those occurring after prolonged hypoglycemia caused by excessive doses of insulin or protamine zinc insulin.

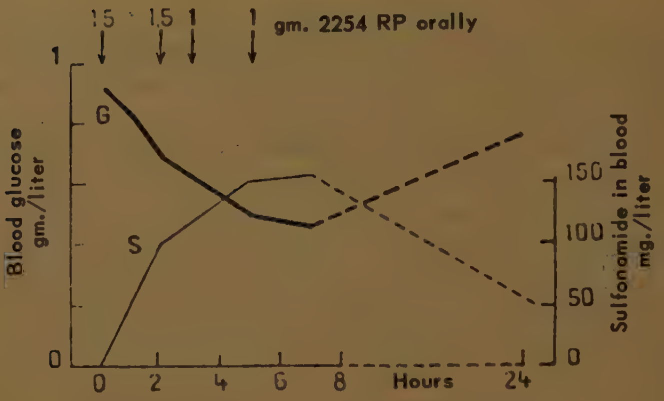

FIGURE 3. Effects of oral administration of repeated doses of 2254 RP (0.31 gm./kg. in all) on the blood-glucose level of a dog. Comparison with the corresponding blood sulfonamide concentration. (Loubatières, 1946a.)

Pancreases from animals (rats, rabbits, and dogs) treated with 2254 RP were examined histologically after staining by Gomori's method. There was considerable diminution of the granulation of the α cells and agglutination of the remaining granules. The relative number of α cells was also decreased.

Effects of 2254 RP in a completely pancreatectomized animal. In a pancreatectomized dog, in contrast to the normal animal, it was not possible to observe the hypoglycemic action of 2254 RP. The presence of the pancreas or of pancreatic tissue is therefore necessary for the manifestation of the hypoglycemic action of this substance.

Acute effects of 2254 RP in an animal that has been partially pancreatectomized or whose pancreas has been experimentally injured. A dog with only a small portion of the pancreas showed the hypoglycemic action of 2254 RP. This effect was also seen in an animal suffering from alloxan diabetes; that is, an animal that still had a few β cells capable of secretory activity. This experiment indicates that 2254 RP stimulates the insulin secretion of the β cells (in agreement with my previous physiological experiments) and that it probably also injures the α cells, reducing their activity even before any serious change is histologically evident.

Effects of chronic administration of 2254 RP to animals with alloxan diabetes. In the experiments on rabbits, the animals received an intravenous alloxan injection of 150 mg./kg. weight. After these animals had been diabetic for 10 to 15 days, the average blood-glucose level was 3 gm./liter and the urinary glucose level 15 to 20 gm./day on a diet composed of oats, carrots, and green leaves. The oral or intramuscular administration of 2254 RP in doses of 0.5 to 1 gm./kg./day (given in 2 parts) for a week, produced progressive decrease and disappearance of the glycosuria; a decrease in the blood-sugar level to normal; a rapid cessation of the weight decrease; and even, in many cases, a weight increase. In most cases there was a permanent cure of the diabetes (FIGURE 4). A second 2254 RP treatment was necessary only if diabetes was progressively re-established after the 2254 RP treatment was stopped (FIGURE 2).

The pancreatic biopsies, after staining by Gomori's method, showed that the β cells were injured (classical alloxan lesions) and the α cells were less numerous and were extensively degranulated. In the pancreas of these animals "cured" of diabetes, there were newly formed islets, of abnormal appearance, as well as acino-insular transition forms.

In the experiments on dogs, 20 animals, weighing from 8 to 15 kg., were made diabetic by an intravenous injection of 50 to 60 mg. alloxan per kg. They were placed on a fixed diet consisting of 200 gm. lean meat and 100 gm. bread per day for a dog weighing 8 kg. Treatment with 2254 RP (0.5 to 1.0 gm./kg./day) was started 8 to 15 days after diabetes appeared. Our research showed that the blood-sugar level was reduced consistently and rapidly; that the glycosuria fell in a parallel manner; that the weight loss was checked; and that some of the animals increased in weight. Treatment was usually continued for from 5 to 7 days, but sometimes for only 36 hours.

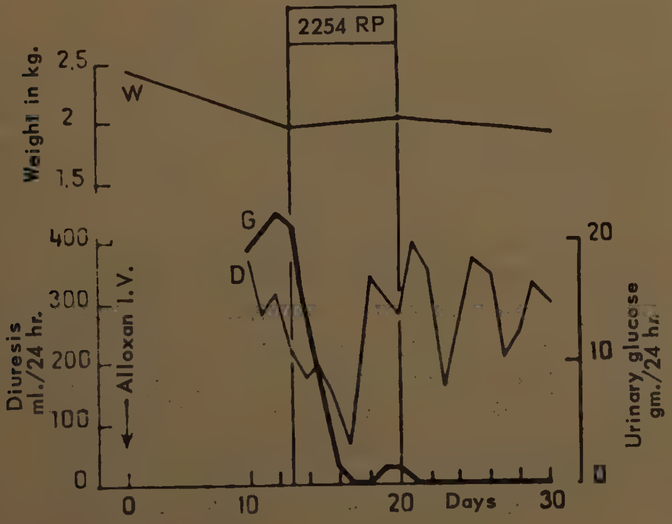

FIGURE 4. Rabbit with alloxan diabetes (7 days' treatment). Effects of the intramuscular injection of 2254 RP (1 gm./kg. for 2 days and 0.5 gm./kg. thereafter). Permanent cure of diabetes. Symbols: W = weight; D = diuresis; G = glycosuria. (Loubatières, Bouyard, and Fruteau de Laclos, 1955.)

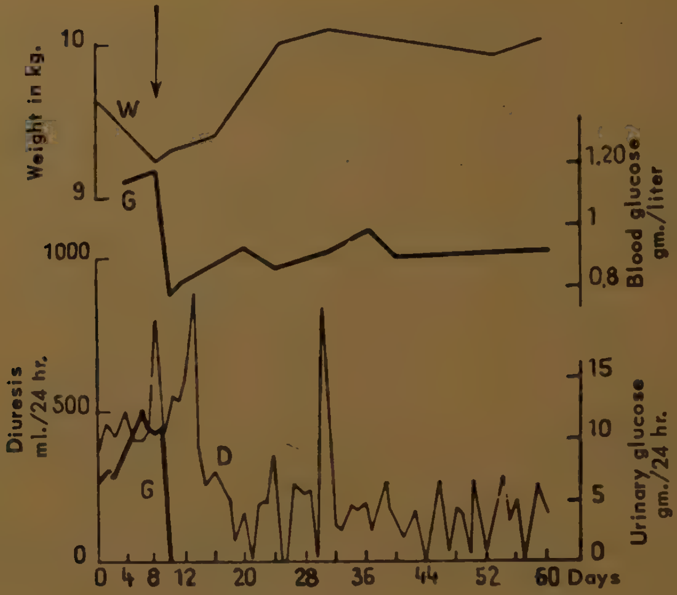

FIGURE 5. Dog with alloxan diabetes. Effect of high doses of 2254 RP (2.5 gm./kg. in 36 hours, administered in 4 portions). Permanent cure of diabetes. In the upper part of the figure, *W* = weight, *G* = blood glucose level; in the lower part of the figure, *D* = diuresis, *G* = glycosuria.

In mild diabetes of recent origin (fasting blood glucose 18 hours after the last meal, around 1.2 gm./liter) we noticed that after large doses of 2254 RP (2.5 gm./kg. given in 4 parts over 36 hours) the blood sugar became normal, the glycosuria disappeared, and the weight decrease was arrested. The diabetic symptoms did not reappear. One animal has remained symptom-free for over 2 months, and there is no reason to suppose that there will be a relapse (FIGURE 5).

In serious diabetes (fasting blood glucose around 3 gm./liter), it was necessary to administer 2254 RP for a greater length of time before the diabetic symptoms began to decrease. With each increase in the dose of 2254 RP there was a corresponding stepwise decrease in the degree of diabetes. In some animals the disease was stabilized at this stage; in others there was progressive re-establishment of the diabetes. As yet we have not observed a complete disappearance of the symptoms in the cases of serious diabetes.

From these experiments it appears that 2254 RP is capable of reducing or curing the dog's alloxan diabetes, if it is recently established or of moderate intensity. In rabbits, however, 2254 RP appears capable of curing even the most serious cases. This difference is probably due to species differences in the reaction capacities of the cells of the islets of Langerhans.

The compound 2254 RP does not seem to have any tachyphylactic action. It is capable of affecting the α and β cells on second administration.

Our recent experiments seem to confirm our hypotheses on the mechanism of action of the drug in the pancreas: (1) a second administration of a diabetogenic dose of alloxan to an alloxan-treated rabbit cured by 2254 RP (free of glycosuria for 20 days) produced a relapse of the diabetes that could again be cured by further treatment with 2254 RP; (2) total pancreatectomy performed on a dog whose alloxan diabetes had been cured by 2254 RP caused a reappearance of the diabetes.

The significance of our experimental work may be summarized as follows:

Physiologically, our experiments appear to provide arguments in favor of the physiological role of the α cells in the regulation of blood sugar.

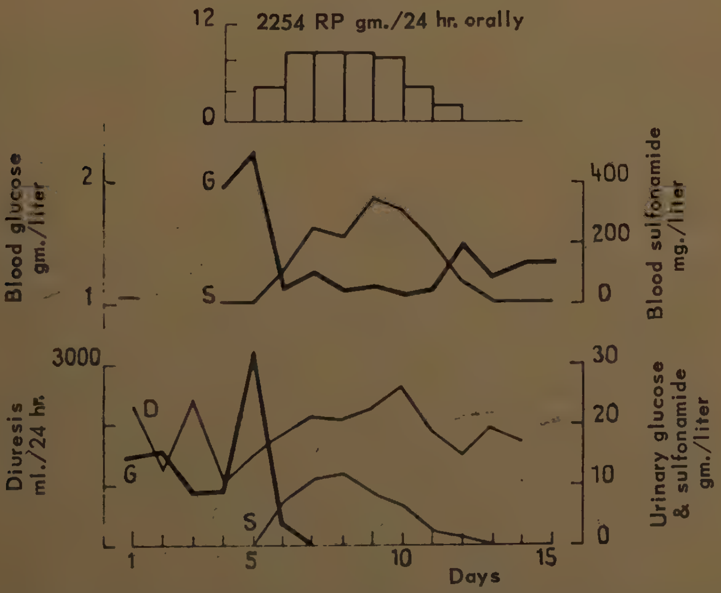

FIGURE 6. Diabetic woman previously treated with insulin. Treatment with 2254 RP for one week. No insulin was given during the entire period of observation. Effects of 2254 RP: disappearance of the glycosuria, normalization of the blood glucose level, disappearance of the ketonuria. In the upper part of the figure, *G* = blood glucose level, *S* = blood-sulfonamide concentration; in the lower part of the figure, *G* = glycosuria, *D* = diuresis, *S* = blood-sulfonamide concentration. (Loubatières, Bouyard, and Fruteau de Laclos, 1955b.)

Pathologically, they seem to show that when diabetes is a result of functional or organic changes in the β cells, changes in the α cells can create a new state of glycemic equilibrium, the stability and duration of which are being studied. In certain cases, the new equilibrium is a result, not only of α-cell lesions, but also of increased functional activity of the β cells. In some species (the rabbit in particular) several relatively well-preserved complexes of β cells can be seen after the administration of curative doses of 2254 RP. We think, however, that possibly in the "permanent" cure of diabetes by 2254 RP, at least in certain species, the phenomenon of general cellular enzymatic adaptation may be involved, as I have suggested earlier (Hédon and Loubatières, 1949, p. 145).

Pharmacologically, these experiments seem to indicate a method of selectively and permanently injuring the α cells of the islets of Langerhans. They open up new fields in the pharmacological treatment of diabetes.

After the animal experiments had been carried out, the last step remained to be taken. Could the results obtained for rabbits and dogs be extrapolated and used on the human being?

Recent Clinical Observations

Very recently we have been able to show with certainty that the hypoglycemic and antidiabetic sulfonamides (as we called them in 1946) will, in the near future, be applied to the treatment of diabetes. Our clinical trials on 2254 RP are encouraging (Loubatières, 1955b; see FIGURE 6).

In October 1955, Franke and Fuchs (1955), Achelis and Hardebeck (1955), and Bertram, Bendfeldt, and Otto (1955) reported the advantages that diabetic patients have gained from their clinical work based on the physiological, histological, and pharmacodynamic experiments carried out by their predecessors and by themselves. The substance they used contains the sulfonamide group, to which is attached a hydrocarbon side chain, the butyl radical, the activity of which I had proved in 1946 (Loubatières, 1946a and c). The number of observations carried out by the German workers is most impressive. Attentive study of their reports, however, shows that the facts reported by these authors do not differ fundamentally from those we had observed ourselves in the diabetic man.

All of these clinical experiments, wherever they have been performed, are very gratifying to us, since they constitute a logical extrapolation of the research carried out in our laboratory for over 13 years on the physiology and the pharmacodynamics of hypoglycemic and antidiabetic sulfonamide derivatives.

Note. This paper was prepared in November 1955. Since that time, further clinical and experimental observations have been published by the author and his collaborators: Boulet *et al.*, 1956; Loubatières, 1955c and d; 1956a to 1956f; Loubatières, Bouyard, and Fruteau de Laclos, 1955f and g; Loubatières *et al.*, 1956a to 1956g.

References

ACHELIS, J.D. & K. HARDEBECK. 1955. A new hypoglycemic substance, preliminary communication. Deut. med. Wochschr. **80:** 1452.

BERTRAM, F., E. BENDFELDT & H. OTTO. 1955. An active oral antidiabetic drug (BZ 55). Deut. med. Wochschr. **80:** 1455.

BOULET, P., A. LOUBATIÈRES, J. MIROUZE, C. FRUTEAU DE LACLOS & P. BOUYARD. 1956. Essais thérapeutiques du diabète sucré humain à l'aide de trois dérivés sulfamidés hypoglycémiants et anti-diabétiques dérivés du *p*-aminobenzène-sulfamido-thiodiazol (isopropyl; isobutyl; tertiobutyl). A propos de cent cas. Comm. 2e réunion scientifique Assoc. Diabétologues de langue francaise, Paris. Le Diabète. To be published.

BOVET, D. & P. DUBOST. 1944. Activité hypoglycemiante des aminobenzenesulfamidoalkylthiodiazols. Rapports entre la constitution et l'activité pharmacodynamique. Compt. rend. soc. biol. **138:** 764.

CHEN, K. K., R. C. ANDERSON & N. MAZE. 1946. Hypoglycemic action of sulfanilamido-cyclopropylthiazole in rabbits, and its reversal by alloxan. Proc. Soc. Exptl. Biol. Med. **63:** 483.

CREUTZFELDT, W. & E. TECKLENBORG. 1955. Synthalin hypoglycemia, α cells and glucagon. Klin. Wochschr. **33:** 43.

DAVIS, J. C. 1952. Hydropic degeneration of the α cells of the pancreatic islets produced by synthalin. J. Pathol. Bacteriol. **64:** 575.

FRANKE, H. & J. FUCHS. 1955. A new antidiabetic principle. Deut. med Wochschr. **80:** 1449.

HÉDON, L. & A. LOUBATIÈRES. 1949. Les diabètes expérimentaux. Rapport, 2ème Congr. Intern. Thérap. Bruxelles. :131-151. Arscia, Bruxelles, Belgium.

HÉDON, L., A. LOUBATIÈRES & G. HEYMANN. 1939. Etude des effets de l'insuline protamine zinc chez le chien dépancréaté. Ann. endocrinol. Paris. **1:** 97.

VON HOLT, C. & H. FERNER. 1955. Morphology of the destruction of the α cells and islets of the pancreas. Z. Zellforsch. **42:** 305.

VON HOLT, C., L. VON HOLT, B. KRÖNER & J. KÜHNAU. 1954. Chemical elimination of the α cells of the islets of Langerhans. Naturwissenschaften. **41:** 166.

VON HOLT, C., L. VON HOLT, B. KRÖNER & J. KÜHNAU. 1955. Effects of chemical elimination of the α cells of the islets of Langerhans on alloxan diabetes. Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol. **224:** 66, 78.

HOUSSAY, B. A. 1950. Action of sulphur compounds on carbohydrate metabolism and on diabetes. Am. J. Med. Sci. **219:** 353.

JANBON, M., J. CHAPTAL & A. VEDEL 1942. Accidents nerveux irréversibles par hypoglycémie due à un sulfamidé : le sulfa-isopropyl-thiodiazol (VK 57 ou 2254 R. P.). Comm. 43ème Congr. Médecins aliénistes et neurologistes de France et des pays de langue francaise, Montpellier. Masson, Ed. Paris, France.

JANBON, M., J. CHAPTAL, A. VEDEL & J. SCHAAP. 1942. Accidents hypoglycémiques graves par un sulfamidothiazol (le VK 57 ou 2254 R.P.). Montpellier méd. **21-22:** 441.

JANBON, M., P. LAZERGES & J. H. METROPOLITANSKI. 1942. Etude du metabolisme du sulfa-isopropyl-thiodiazol (VK 57 ou 2254 R. P.) chez le

sujet sain et en cours de traitement. Comportement de la glycémie. Montpellier méd. **21-22**: 489. Comm. Soc. Sci. méd.biol. Montpellier de la clinique des Maladies Contagieuses. (1941-1942). : 212. Ch. Déhan. Montpellier, France.

JANBON, M., P. LAZERGES, J. H. METROPOLITANSKI & J. D. SCHAAP. 1943. Action hypoglycémiante de certains composés sulfamidés. Rôle du radical propylthiodiazol. Montpellier Méd. **23-24**: 132.

LOUBATIÈRES, A. 1938. Insuline et fonction glycogénique du foie. Contribution à l'étude du mécanisme d'action de la sécrétion interne du pancréas. Thèse Doct. Méd. Montpellier. 100 pp. E. Mazel, Ed. Largentières, France.

LOUBATIÈRES, A. 1944a. Analyse du mécanisme de l'action hypoglycémiante du *p*-amino-benzène-sulfamido-isopropyl-thiodiazol (2254 R. P.). Compt. rend. soc. biol. **138**: 766.

LOUBATIÈRES, A. 1944b. Relations entre la structure moléculaire et l'activité hypoglycémiante des aminobenzène-sulfamido-alkylthiodiazols. Compt. rend. soc. biol. **138**: 830.

LOUBATIÈRES, A. 1946a. Physiologie et pharmacodynamie de certains dérivés sulfamidés hypoglycémiants. Contribution à l'étude des substances synthétiques à tropisme endocrinien. Thèse Doct. Sci. naturelles. Montpellier. No. 86. Causse, Graille et Castelnau, Ed. Montpellier, France.

LOUBATIÈRES, A. 1946b. Etude experimentale du diabète déterminé par alloxanisation (diabète de Dunn) chez le lapin et le chien. Arch. intern. physiol. **54**: 170.

LOUBATIÈRES, A. 1946c. Etude physiologique et pharmacodynamique de certains dérivés sulfamidés hypoglycémiants. Arch. intern. physiol. **54**: 174.

LOUBATIÈRES, A. 1946d. Les dérivés sulfamidés hypoglycémiants. Etude physiologique et pharmacodynamique.45ème Congr. Assoc. Française.Avancement Sci. (Section Pharmacologie). Nice, France. (Abstr., Presse Médicale, 1946, **54**: 754.)

LOUBATIÈRES, A. 1952a. Acquisitions dans le domaine de la pharmacologie du diabète sucré. Maroc Méd. **1952**:(323) 361.

LOUBATIÈRES, A. 1952b. Orientation pharmacologique des recherches modernes sur le diabète sucré. Conf. Fac. Méd. Paris, France. *In* Actualités Pharmacologiques. R. Hazard, Ed. 5ème série, 186-211. Masson Ed. Paris.

LOUBATIÈRES, A. 1954. Does alloxan play a part in the pathogenesis of diabetes mellitus? *In* Experimental Diabetes and Its Relation to the Clinical Disease. Blackwell Scientific Publ. Oxford, England.

LOUBATIÈRES, A. 1955a. L'utilisation de certaines substances sulfamidées dans le traitement du diabète sucré expérimental. Recherches personnelles (1942-1946). Presse méd. **63**: 1701. Les substances sulfamidees dans le traitement du diabète sucré. Recherches personnelles (1946-1955). Confirmations expérimentales et recents developpements. Presse méd. **63**: 1728.

LOUBATIÈRES, A. 1955b. Action curative du *p*-aminobenzène-sulfamido-isopropyl-thiodiazol dans le diabète sucré humain. Compt. rend. acad. Sci. **241**: 1422.

LOUBATIÈRES, A. 1955c. Utilisation de substances sulfamidées dans le traitement du diabète sucré. Historique et antériorités. Thérapie. **10**: 907.

LOUBATIÈRES, A. 1955d. Historique et développements récents dans le domaine des sulfamidés hypoglycémiants et antidiabétiques. Med. Hyg. **13**: 495.

LOUBATIÈRES, A. 1956a. Historique et mécanisme d'action des sulfamidés hypoglycémiants et antidiabétiques. J. Diabétiques. **10**: 9.

LOUBATIÈRES, A. 1956b. Acquisitions récentes dans le domaine de l'expérimentation des sulfamidés hypoglycémiants et antidiabétiques chez l'animal et chez l'Homme diabétiques. Thérapie. In press.

LOUBATIÈRES, A. 1956c. Action hypoglycémiante et antidiabétique du *p*-aminobenzène-sulfamido-tertiobutyl-thiodiazol. Compt. rend acad. sci. **243**: 420.

LOUBATIÈRES, A. 1956d. Action hypoglycémiante et antidiabétique de certains sulfamidés. Abstracts, XXe Congr. Intern. Physiol. Bruxelles, Belgium. : 590.

Loubatières, A. 1956e. Der Wirkungsmechanismus der blutzuckersenkenden Sulfonamide und Antidiabetika im Lichte der jüngsten experimentellen und klinischen Befunde. Comm. Deutsche Therapiewoche. (Therapiekongress). Karlsruhe, Germany. In press.

Loubatières, A. 1956f. Le mécanisme d'action des dérivés sulfamidés hypoglycémiants et antidiabétiques à la lumière des expériences récentes. Comm. 2e réunion scientifique Assoc. Diabétologues de langue française, Paris, France. Le Diabète. In press.

Loubatières, A. & P. Bouyard. 1951. Inhibition de la formation d'alloxane *"in vitro"* et *"in vivo"* chez l'animal normal ou diabétique. J. Physiol. (Paris). **43**: 786.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1954. Effets de l'hyperpression liquidienne dans les canaux pancréatiques sur la glycémie du chien chloralosé. Compt. rend soc. biol. **148**: 2056.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955a. Effets sur la glycémie de l'injection d'alloxane dans le canal de Wirsung du chien chloralosé. Compt. rend. soc. biol. **149**: 162.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955b. La distension du système excréteur pancréatique est-elle susceptible de modifier la régulation glycémique? J. Physiol. (Paris). **47**: 227.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955c. Actions du *para*-aminobenzène-sulfamido-isopropyl-thiodiazol sur la glycémie, la structure des ilôts de Langerhans et le métabolisme de l'animal normal ou diabétique. Compt. rend. soc. biol. **149**: 1642.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955d. Analyse du mécanisme de l'action curatrice que le *para*-aminobenzène-sulfamido-isopropyl-thiodiazol exerce sur le diabète sucré expérimental provoqué par l'alloxane. Compt. rend. acad. Sci. **241**: 515.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955e. Action curatrice du *para*-aminobenzène-sulfamido-isopropyl-thiodiazol sur le diabète sucré expérimental. Analyse du mécanisme d'action de cette substance. Comm. 1ère réunion Assoc. Diabétologues Langue Française, Vals-les-Bains. Le Diabète. **4**: 38.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955f. Arguments physiologiques récents en faveur de l'origine intrapancréatique de l'action hypoglycémiante et antidiabétique du *p*-aminobenzène-sulfamido-isopropyl-thiodiazol. Compt. rend. soc. biol. **149**: 2187.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1955g. Effets chez l'Homme diabétique du *p*-aminobenzène-sulfamido-isopropylthiodiazol. Montpellier méd. **48**: 618.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1956a. Etude expérimentale chez le diabétique humain du *p*-aminobenzène-sulfamido-isopropyl-thiodiazol. Sem. Hôp. Paris. (Ann. Rech. Med.). **32**: 47.

Loubatières, A., P. Bouyard & C. Fruteau de Laclos. 1956b. Arguments en faveur de l'origine pancréatique de l'action hypoglycémiante et antidiabétique du *p*-aminobenzène-sulfamido-isopropyl-thiodiazol. J. Physiol. (Paris). **48**: 618.

Loubatières, A., P. Bouyard, C. Fruteau de Laclos & A. Sassine. 1956c. Origine intrapancréatique de l'action hypoglycémiante et antidiabétique du *p*-amino-benzène-sulfamido-isopropyl-thiodiazol. Compt. rend acad. sci. **242**: 2044.

Loubatières, A., P. Bouyard, C. Fruteau de Laclos & A. Sassine. 1956d. L'hypophyse et les centres diencéphaliques sont-ils directement impliqués dans l'action hypoglycémiante du *p*-aminobenzène-sulfamido-isopropyl-thiodiazol? Compt. rend soc. biol. **150**: 770.

Loubatières, A., P. Bouyard, C. Fruteau de Laclos & A. Sassine. 1956e. Hypophyse et mécanisme d'action du *p*-aminobenzène-sulfamido-isopropyl-thiodiazol. J. Physiol. (Paris). **48**: 620.

Loubatières, A., P. Bouyard, C. Fruteau de Laclos & A. Sassine. 1956f. Etude de l'action hypoglycémiante et antidiabétique du *p*-amino-

benzène-sulfamido-tertiobutyl-thiodiazol chez l'Homme diabétique. Compt. rend. soc. biol. In press.

LOUBATIÈRES, A., P. BOUYARD, C. FRUTEAU DE LACLOS, A. SASSINE & R. ALRIC. 1956g. Renforcement et prolongation des effets de l'insuline par les sulfamidés hypoglycémiants et antidiabétiques. Compt. rend. soc. biol. In press.

LOUBATIÈRES, A. & J. BROUSSY. 1938. Sur quelques particularités des accidents hypoglycémiques présentés par les animaux traités par l'insuline-protamine-zinc. Arch. soc. sci. méd. biol. Montpellier et Languedoc. 8: 399.

LOUBATIÈRES, A., C. FRUTEAU DE LACLOS & P. BOUYARD. 1956. Le *p*-aminobenzène-sulfamido-isopropyl-thiodiazol dans le traitement du diabète sucré. Observations cliniques et essai d'interprétation concernant le mécanisme d'action de cette substance. Sem. Hôp. Paris. 32: 2358.

LOUBATIÈRES, A., L. GOLDSTEIN, J. METROPOLITANSKI & J. SCHAAP 1942. Etude expérimentale chez le chien des accidents nerveux irréversibles consécutifs à l'hypoglycémie prolongée provoquée par la sulfa-isopropyl-thiodiazol. Comm. 43ème Congr. Méd. aliénistes et neurol. France et des pays de langue francaise, Montpellier : 415. Masson Ed. Paris, France.

LOUBATIÈRES, A. & G. HEYMANN. 1938. Etude expérimentale des effets de l'insuline-protamine-zinc chez le chien dépancréaté. Arch. soc. sci. méd. biol. Montpellier et Languedoc. 8: 393.

METROPOLITANSKI, J. H. Comportement *in vivo* d'un dérivé sulfamido-thiazolé (2254 R. P.). Thèse Doct. Méd. Montpellier. Ch. Déhan. Montpellier, France.

SCHAAP, J. D. 1942. Essais thérapeutiques avec un sulfamidothiodiazol (2254 R. P.). Thèse Doct. Med. Montpellier, Ch. Déhan. Montpellier, France.

Printed in the United States of America

MONOGRAPHIC PUBLICATIONS

OF

THE NEW YORK ACADEMY OF SCIENCES

(Lyceum of Natural History, 1817–1876)

(1) The Annals (octavo series), established in 1823, contain the scientific contributions and reports of researches, together with the records of meetings of the Academy. The articles which comprise each volume are printed separately, each in its own cover, and are distributed immediately upon publication. The price of the separate articles depends upon their length and the number of illustrations, and may be ascertained upon application to the Executive Director of the Academy.

Current numbers of the Annals are sent free to all Members of the Academy desiring them.

(2) The Special Publications established in 1939, are issued at irregular intervals as cloth-bound volumes. The price of each volume will be advertised at time of issue.

(3) The Memoirs (quarto series), established in 1895, are issued at irregular intervals. It is intended that each volume shall be devoted to monographs relating to some particular department of science. Volume I, Part 1 is devoted to Astronomical Memoirs, Volume II to Zoological Memoirs. No more parts of the Memoirs have been published to date. The price is one dollar per part.

(4) The Scientific Survey of Porto Rico and the Virgin Islands (octavo series), established in 1919, gives the detailed reports of the anthropological, botanical, geological, paleontological, zoological, and meteorological surveys of these islands.

Subscriptions and inquiries concerning current and back numbers of any of the publications of the Academy should be addressed to

EXECUTIVE DIRECTOR
The New York Academy of Sciences
2 East Sixty-third Street
New York 21, N. Y.